Neuropharmacology 91 (2015) 142–147

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Tasimelteon: A selective and unique receptor binding profile

Christian Lavedan [a, *], Mark Forsberg [a], Anthony J. Gentile [b]

[a] Vanda Pharmaceuticals Inc., 2200 Pennsylvania Ave. N.W., Suite 300-E, Washington, D.C, 20037, USA
[b] Bristol-Myers Squibb, 5 Research Parkway, Wallingford, CT, 06492, USA

ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form
24 November 2014
Accepted 2 December 2014
Available online 19 December 2014

Chemical compounds studied in this article:
Tasimelteon (PubChem CID:10220503)

Keywords:
Tasimelteon
Hetlioz®
Circadian rhythm
Circadian regulator
Non-24
Melatonin receptor

ABSTRACT

Hetlioz® (tasimelteon) is the first approved treatment in the United States for Non-24-Hour Sleep-Wake Disorder (Non-24). We present here data on the *in vitro* binding affinity of tasimelteon for both human melatonin receptors MT_1 and MT_2, as well as the extended screen of other receptors and enzymes. Results indicate that tasimelteon is a potent Dual Melatonin Receptor Agonist (DMRA) with 2.1–4.4 times greater affinity for the MT_2 receptor believed to mediate circadian rhythm phase-shifting (K_i = 0.0692 nM and K_i = 0.17 nM in NIH-3T3 and CHO–K1 cells, respectively), than for the MT_1 receptor (K_i = 0.304 nM and K_i = 0.35 nM, respectively). Tasimelteon was also shown to have no appreciable affinity for more than 160 other pharmacologically relevant receptors and several enzymes.

1. Introduction

Circadian rhythms are regulated by an endogenous circadian pacemaker that resides in the suprachiasmatic nuclei (SCN) in mammals. This pacemaker is the Master Body Clock that regulates the circadian rhythms of biological processes including the secretion of hormones such as melatonin and cortisol, sleep-wake cycles, alertness and performance patterns, metabolism and cardiovascular processes (Lockley et al., 2007, 2008). In human, the endogenous rhythm of the SCN is typically slightly longer than 24 h and therefore must be entrained (synchronized) to the 24-h day. The strongest zeitgeber responsible for entrainment is the daily light–dark cycle, detected primarily by the intrinsically photosensitive ganglion cells of the retina (Czeisler and Gooley, 2007; Ramsey et al., 2013).

Disruptions in a person's circadian rhythms have been recognized to play a significant role in the etiology of a number of serious and chronic disorders, including obesity (Karlsson et al., 2001), diabetes (Morikawa et al, 2005), breast cancer (Davis et al., 2001; Schernhammer et al, 2001), colorectal cancer (Schernhammer et al, 2003), cardiovascular diseases (Vyas et al, 2012; Ruger and Scheer, 2009; Knutsson, 2003), depression (Boyce and Barriball, 2010; Monteleone and Maj, 2008; Turek, 2007), and circadian-rhythm sleep-wake disorders (Sack et al, 2007) like Non-24-Hour Sleep-Wake Disorder (Non-24) (Lockley et al., 2007; Skene et al., 1999). Non-24 is a serious, debilitating, chronic disorder that occurs when individuals are unable to synchronize their endogenous circadian clock to the 24-h light-dark cycle (American Psychiatric Association, 2013; American Academy of Sleep Medicine, 2005). The majority of reported cases of Non-24 occur in blind patients with no conscious perception of light (Lockley et al., 2007; Uchiyama and Lockley, 2009). The growing understanding of the importance of circadian regulation in these complex disorders is expected to spawn the development of specifically designed circadian regulators that can address the underlying circadian component of these conditions.

Abbreviations: AMP, Adenosine monophosphate; BSA, Bovine serum albumin; cAMP, Cyclic AMP; DMRA, Dual Melatonin Receptor Agonist; EC_{50}, 50% of the maximal effect; EDTA, Ethylenediaminetetraacetic acid; E_{max}, Maximal effect; GABA, Gamma-aminobutyric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IBMX, 3-isobutyl-1-methylxanthine; IC_{50}, Concentration producing 50% of the maximal effect; K_i, Dissociation constant for the inhibitor; NMDA, N-methyl-D-aspartate; Non-24, Non-24-Hour Sleep-Wake Disorder; PKC, Protein kinase C; PMSF, phenylmethylsulfonyl fluoride; SCN, Suprachiasmatic nuclei; TRIS, Tris(hydroxymethyl)aminomethane; 4-P-PDOT, Cis-4-Phenyl-2-propionamidotetralin(.

* Corresponding author. Tel.: +1 202 764 3400; fax: +1 202 296 1450.
E-mail address: Christian.lavedan@vandapharma.com (C. Lavedan).

http://dx.doi.org/10.1016/j.neuropharm.2014.12.004

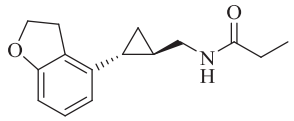

Fig. 1. Chemical structure of tasimelteon.

Tasimelteon is a novel structurally unique molecular entity, chemically designated as (1R, 2R)-N-[2- (2,3-dihydrobenzofuran-4-yl)cyclopropylmethyl]propanamide, containing two chiral centers that was developed for the treatment of Non-24. Its molecular formula is $C_{15}H_{19}NO_2$, and the molecular weight is 245.32. Tasimelteon differs structurally from melatonin and drugs with known melatonin agonist activity, in particular by its distinct aromatic group and linker (Fig. 1). Tasimelteon bears also no structural relationship to any other approved active substance.

Tasimelteon was specifically developed to treat the underlying circadian basis of Non-24, and has undergone rigorous safety evaluation (Swick et al., 2014; Sliman et al., 2014; Quera-Salva et al., 2014; Lockley et al., unpublished results). Non-24 is a circadian rhythm disorder, which primarily affects totally blind individuals, characterized by the inability to entrain the master body clock to the 24-h light–dark cycle (American Psychiatric Association, 2013; American Academy of Sleep Medicine, 2005). It is estimated that seventy percent of blind individuals with no light perception have Non-24 (Sack and Lewy, 2001). Patients with Non-24 have prolonged periods of misalignment of circadian rhythms, including the timing of melatonin and cortisol secretion and the sleep-wake cycle, which are associated with significant impairments in social and occupational functioning, and marked subjective distress (American Psychiatric Association, 2013).

Studies reported here investigated the binding characteristics of tasimelteon for a wide range of receptors and other pharmacological targets, in particular the G protein-coupled melatonin MT_1 and MT_2 receptors, which are expressed in the central nervous system and in peripheral tissues (Dubocovich and Markowska, 2005). Results point to the unique receptor binding profile of tasimelteon, which acts as a selective and potent DMRA with greater affinity for the MT_2 receptor than for the MT_1 receptor, and no appreciable affinity for more than 160 other pharmacologically relevant receptors and enzymes, including the gamma-aminobutyric acid (GABA) receptor complex and receptors that bind neuropeptides, cytokines, serotonin, noradrenaline, acetylcholine, and opiates.

Table 1
Binding affinity of tasimelteon at the human melatonin receptors.

Cell system	K_i (nM)		K_i ratio
	MT_1	MT_2	MT_1/MT_2
NIH-3T3	0.35	0.17	2.1
CHO–K1	0.304	0.0692	4.4

2. Materials and methods

The specific affinity of tasimelteon for the MT_1 and MT_2 receptors was evaluated in two independent experiments using human recombinant receptors and radioligand binding assays. The selectivity of the receptor binding was further evaluated by determining the affinity for more than 160 other receptors and several enzymes. Furthermore, inhibition of forskolin-stimulated cyclic adenosine monophosphate (cyclic AMP or cAMP) accumulation was conducted in NIH-3T3 cells stably expressing the human MT_1 and MT_2 receptors.

2.1. Binding assays at the MT_1 and MT_2 receptors

In a first experiment, two lines of NIH-3T3 cells were used; one cell line stably expressed the human MT_1 receptor while the second cell line stably expressed the human MT_2 receptor. Cells were maintained and passaged as attached monolayers in Dulbecco's Modified Eagle Medium containing D-glucose, 10 mM HEPES, 10% heat inactivated calf serum and 500 μg/mL geneticin. Cultures were incubated in vented tissue culture flasks at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were harvested and frozen as pellets at −80 °C. Membrane homogenates were prepared in assay buffer (50 mM TRIS containing 12.5 mM $MgCl_2$ and 2 mM EDTA, pH 7.4 at 37 °C with HCl) fortified with 10 μg/mL aprotinin and leupeptin and 100 μM PMSF, using a Dounce homogenizer. The resulting homogenate was centrifuged (45,000 × *g*, 10 min, 4 °C) and the pellet was resuspended in fortified assay buffer at a ratio of 0.25 mL per original flask of cells and frozen in aliquots at −80 °C until use. Binding assays were performed in duplicate in a total volume of 0.2 mL containing 0.16 mL membrane homogenate diluted appropriately, 0.02 mL 2-[^{125}I]iodomelatonin (0.1 or 0.2 nM final concentration for MT_1 or MT_2, respectively) and 0.02 mL assay buffer, melatonin or tasimelteon. Nine concentrations of tasimelteon (0.001 nM, 0.01 nM, 0.03 nM, 0.1 nM, 0.3 nM, 1 nM, 3 nM, 10 nM, and 100 nM) and 5 concentrations of melatonin (0.01 nM, 0.1 nM, 1 nM, 10 nM, and 100 nM) were used for each MT_1 or MT_2 binding assay. Non-specific binding was determined in the presence of 10 μM melatonin. Assays were initiated by the addition of membrane

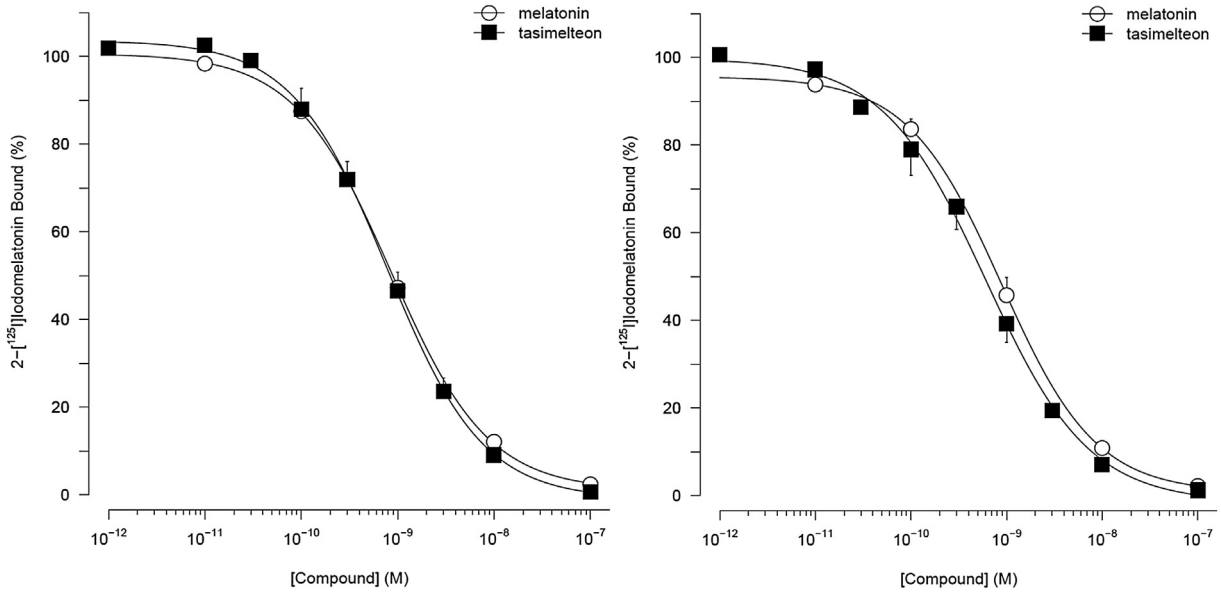

Fig. 2. Affinity of tasimelteon at the human melatonin receptors MT_1 and MT_2 in NIH-3T3 cells. Data used for the non-linear regression analysis are shown as the mean ± standard error of the mean for the MT_1 receptor (left graph) and for the MT_2 receptor (right graph) stably expressed in NIH-3T3 cells.

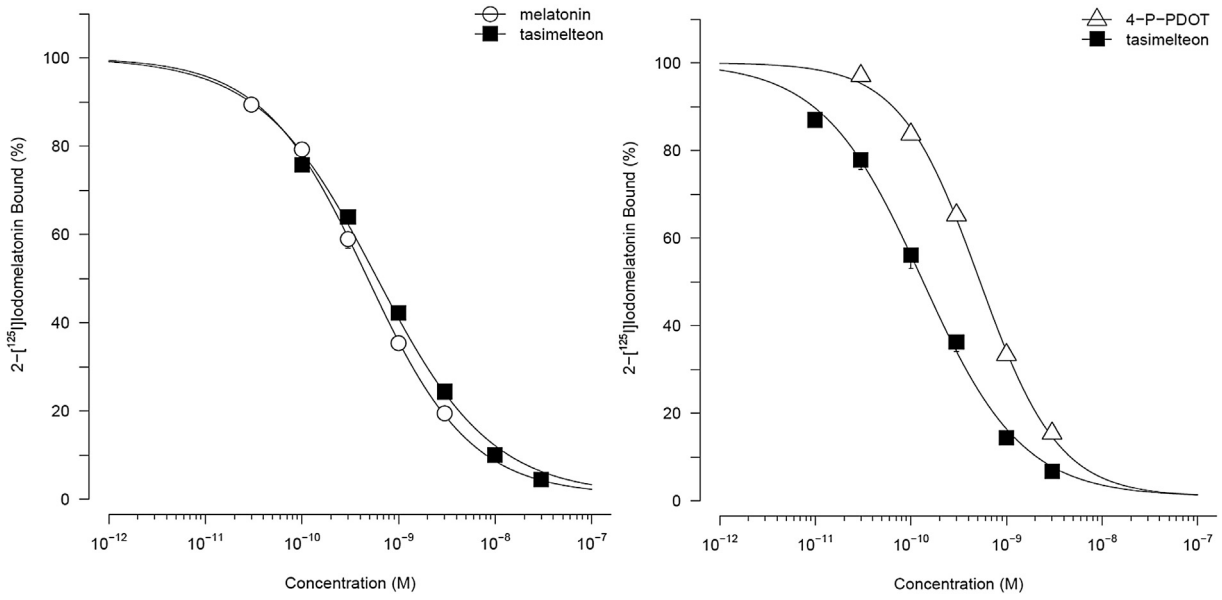

Fig. 3. Affinity of tasimelteon at the human melatonin receptors MT_1 and MT_2 in CHO–K1 cells. Data used for the non-linear regression analysis are shown as the mean ± standard error of the mean for the MT_1 receptor (left graph) and for the MT_2 receptor (right graph) expressed in CHO–K1 cells.

homogenates and incubated for 60 min at 37 °C. Assays were terminated by rapid filtration onto Whatman GF/B filters with a Brandel cell harvester. Filters were washed 3 times with 4 mL ice-cold 20 mM TRIS containing 2 mM $MgCl_2$, pH 7.4 at 25 °C. Bound radioactivity was quantitated by gamma emission spectrometry. Data were analyzed by a 4-parameter logistic, non-linear least squares regression (DeLean et al., 1978) to yield IC_{50} values (concentration producing 50% of the maximal effect), which were converted to dissociation constant for the inhibitor (K_i) values (Cheng and Prusoff, 1973).

In a second experiment, two lines of CHO–K1 cells were used; one cell line expressed the human MT_1 receptor while the other expressed the human MT_2 receptor. Radioligand binding assays were performed with 0.05 nM 2-[^{125}I]iodomelatonin used as the ligand, with a 4 h incubation at 25 °C in 25 mM HEPES, pH 7.4, 5 mM MgCl, 1 mM CaCl, 0.1% BSA. 6-Chloromelatonin (1 μM) was used as non-specific ligand. The reference compounds used for the MT_1 or MT_2 binding assays were melatonin and 4-P-PDOT, respectively; 4-P-PDOT was used in this second experiment because it was shown to have substantially higher affinity for the MT_2 receptor than for the MT_1 receptor (Browning et al., 2000). Six concentrations of tasimelteon (0.1 nM, 0.3 nM, 1 nM, and 3 nM, 10 nM and 30 nM for MT_1; 0.01 nM, 0.03 nM, 0.1 nM, 0.3 nM, 1 nM, and 3 nM for MT_2) were used in triplicate batches, with duplicate assays for each batch. IC_{50} values were determined by a non-linear, least squares regression analysis using MathIQ™ (ID Business Solutions Ltd., UK). K_i values were calculated according to the equation of Cheng and Prusoff (Cheng and Prusoff, 1973) using the observed IC_{50} of the tested compound, the concentration of radioligand employed in the assay, and the historical values for the dissociation constant of the ligand (obtained experimentally at MDS Pharma Services).

2.2. Cyclic adenosine monophosphate assays

Cyclic AMP assays were performed using NIH-3T3 cells stably expressing human MT_1 or MT_2 receptors. Cells were incubated in culture media containing 1 mM IBMX and 10 μM forskolin for 10 min at 37 °C in the presence of increasing concentrations (1 pM–10 μM) of melatonin or tasimelteon. Assays were terminated by the addition of 0.1 N HCl. Following centrifugation, supernatants were collected and stored at −20 °C until assayed. Cyclic AMP levels were measured by radioimmunoassay

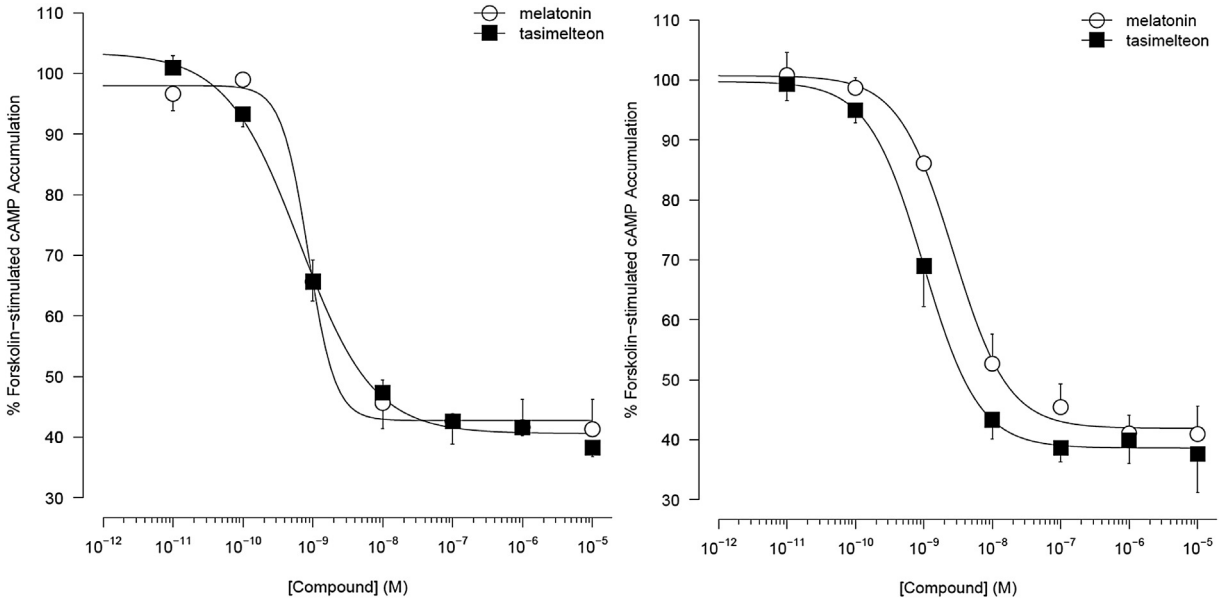

Fig. 4. Inhibition by tasimelteon of forskolin-stimulated cAMP accumulation in NIH-3T3 cells stably expressing human melatonin receptors MT_1 and MT_2. Data used for the non-linear regression analysis are shown as the mean ± standard error of the mean for the MT_1 receptor (left graph) and for the MT_2 receptor (right graph).

Table 2
List of receptors for which tasimelteon (10 μM) did not inhibit or stimulate binding by more than 50%.

Receptor	Receptor	Receptor
Adenosine A_1	$GABA_{B1A}$	Potassium channel [SK_{CA}]
Adenosine A_{2A}	$GABA_{B1B}$	Potassium channel HERG
Adenosine A_3	Gabapentin	Progesterone
Adrenergic α_{1A}	Galanin GAL1	Progesterone PR-B
Adrenergic α_{1B}	Galanin GAL2	Prostanoid CRTH2
Adrenergic α_{1D}	Glucocorticoid	Prostanoid DP
Adrenergic α_2	Glutamate, AMPA	Prostanoid EP_2
Adrenergic α_{2A}	Glutamate, Kainate	Prostanoid EP_4
Adrenergic α_{2C}	Glutamate, NMDA	Prostanoid, thromboxane A_2
Adrenergic β_1	Glycine, strychnine-sensitive	Purinergic P_{2X}
Adrenergic β_2	Growth hormone secretagogue	Purinergic P_{2Y}
Adrenergic β_3	Histamine H_1, central	Retinoid X Receptor RXRα
Adrenomedullin AM_1	Histamine H_2	Rolipram
Adrenomedullin AM_2	Histamine H_3	Ryanodine RyR3
Aldosterone	Histamine H_4	Serotonin 5-HT_1
Anaphylatoxin C5a	Hypocretin (orexin) receptor 1	Serotonin 5-HT_{1A}
Androgen	Hypocretin (orexin) receptor 2	Serotonin 5-HT_{1B}
Angiotensin AT_1	Imidazoline I_2, central	Serotonin 5-HT_2
Angiotensin AT_2	Inositol trisphosphate IP_3	Serotonin 5-HT_{2A}
Apelin (APJ)	Insulin	Serotonin 5-HT_{2B}
Atrial natriuretic factor	Interleukin IL-1	Serotonin 5-HT_{2C}
Bombesin BB1	Interleukin IL-2	Serotonin 5-HT_3
Bombesin BB2	Interleukin IL-6	Serotonin 5-HT_4
Bombesin BB3	Leptin	Serotonin 5-HT_{5A}
Bradykinin B_1	Leukotriene (LTB_4)	Serotonin 5-HT_6
Bradykinin B_2	Leukotriene, Cysteinyl $CysLT_1$	Sigma σ_1
Calcitonin	Leukotriene, Cysteinyl $CysLT_2$	Sigma σ_2
Calcitonin gene-related peptide $CGRP_1$	Melanocortin MC_1	Sodium channel, Site 2
Calcium channel L-type	Melanocortin MC_3	Somatostatin sst1
Calcium channel N-type	Melanocortin MC_4	Somatostatin sst2
Cannabinoid CB_1	Melanocortin MC_5	Somatostatin sst3
Cannabinoid CB_2	Motilin	Somatostatin sst4
Chemokine CCR1	Muscarinic M_1	Somatostatin sst5
Chemokine CCR2B	Muscarinic M_2	Tachykinin NK_1
Chemokine CCR4	Muscarinic M_3	Tachykinin NK_2
Chemokine CCR5	Muscarinic M_4	Tachykinin NK_3
Chemokine CX3CR1	Muscarinic M_5	Thromboxane A_2
Chemokine CXCR2 (IL-$8R_B$)	N-formyl peptide receptor FPR1	Thyroid hormone
Cholecystokinin CCK_1 (CCK_A)	N-formyl peptide receptor- Like FPRL1	Thyrotropin releasing hormone
Cholecystokinin CCK_2 (CCK_B)	Neurokinin NK_1	Transforming growth factor-β
Colchicine	Neuromedin U NMU_1	Transporter, adenosine
Corticotropin releasing factor CRF_1	Neuromedin U NMU_2	Transporter, choline
Dopamine D_1	Neuropeptide Y, Y_1	Transporter, dopamine
Dopamine D_{2L}	Neuropeptide Y, Y_2	Transporter, GABA
Dopamine D_{2S}	Neurotensin NT_1	Transporter, monoamine
Dopamine D_3	Nicotinic acetylcholine	Transporter, norepinephrine
Dopamine $D_{4.2}$	Nicotinic acetylcholine α1,	Transporter, serotonin
Dopamine D_5	Nicotinic acetylcholine α7,	Tumor necrosis factor
Endothelin ET_A	Opiate δ (OP1, DOP)	Urotensin II
Endothelin ET_B	Opiate κ (OP2, KOP)	Vanilloid
Epidermal growth factor	Opiate μ (OP3, MOP)	Vascular endothelial growth factor
Erythropoietin EPOR	Orphanin ORL_1	Vasoactive intestinal peptide
Estrogen ERα	Phorbol ester	Vasoactive intestinal peptide 1
Estrogen ERβ	Platelet activating factor	Vasopressin V_{1A}
G Protein-coupled receptor GPR103	Platelet-derived growth factor	Vasopressin V_{1B}
G Protein-coupled receptor GPR8	Potassium channel [K_A]	Vasopressin V_2
$GABA_B$	Potassium channel [K_{ATP}]	Vitamin D_3

(Amersham). Radioactivity was quantitated by gamma emission spectrometry. Three separate experiments were performed in duplicate. Data were analyzed by a 4-parameter logistic, non-linear least squares regression (DeLean et al., 1978) to yield concentration producing 50% of the maximal effect (EC_{50}) and maximal effect (E_{max}) values.

2.3. Extended binding profile

Standard radioligand binding and enzyme inhibition assays were performed on receptors, binding sites or enzyme systems obtained from various sources, including human, rat, mouse, guinea pig, rabbit, hamster, and bovine tissues (see Supplemental Information), using the ProfilingScreen and DiscoveryScreen panels (Panlabs) which consisted of 56 radioligand binding assays and 7 enzyme assays, respectively, and the SpectrumScreen panel (MDS Pharma Services) that included 170 pharmacological relevant targets (see Supplemental Information). In addition, the $GABA_A$ benzodiazepine and $GABA_B$ binding sites were also tested independently (Panlabs biochemical pharmacology assays). Tasimelteon was used at a concentration of 10 μM except for 2 enzyme assays (Protein kinases C: PKCα and PKCβ) where it was used at 100 μM, and for the melatonin receptors in the SpectrumScreen panel where 4 concentrations (10 nM, 0.1 μM, 1 μM and 10 μM) were tested. A response was considered significant if there was ≥50% inhibition or stimulation for the assays.

The affinity of tasimelteon (10 μM) for the human hypocretin (orexin) receptor 1 expressed in transfected CHO cells, and for the human hypocretin (orexin) receptor 2 expressed in transfected HEK-293 cells, was determined in radioligand binding assays (Eurofins Cerep SA, Celle l'Evescault, France).

3. Results

Tasimelteon potently inhibits 2-[^{125}I]iodomelatonin (0.1 or 0.2 nM) binding at the human MT_1 and MT_2 receptors stably

expressed in NIH-3T3 cells. In this experiment, tasimelteon displayed comparable potency to melatonin at the MT_1 receptor and stronger potency than melatonin ($P < 0.05$) at the MT_2 receptor (Fig. 2). Non-linear regression analysis of the data for the MT_1 receptor yielded pK_i values (±standard error of the mean) of 9.39 ± 0.02 (K_i = 0.41 nM) for melatonin and pK_i = 9.45 ± 0.04 (K_i = 0.35 nM) for tasimelteon (Table 1); for the MT_2 receptor values were pK_i = 9.57 ± 0.03 (K_i = 0.27 nM) for melatonin and pK_i = 9.77 ± 0.07 (K_i = 0.17 nM) for tasimelteon (Table 1). Analysis of similar binding assays performed with CHO–K1 cells confirmed a higher affinity of tasimelteon for the MT_2 receptor, with K_i = 0.304 nM and K_i = 0.0692 ± 0.007 nM for MT_1 and MT_2 respectively (Fig. 3, Table 1). In CHO–K1 cells, positive controls melatonin and 4-P-PDOT had K_i values of 0.246 ± 0.039 nM and 0.273 ± 0.023 nM for MT_1 and MT_2, respectively. Binding kinetics were best fit to a one-site binding model, suggesting a single receptor interaction. Taken together, results indicate that tasimelteon is a DMRA with 2.1–4.4 times greater affinity for the MT_2 receptor than for the MT_1 receptor (Table 1). For melatonin, affinity for the MT_2 receptor was 1.5 fold higher than for the MT_1 receptor in NIH-3T3 cells.

Tasimelteon was shown to induce a potent, concentration-dependent inhibition of forskolin-stimulated cAMP accumulation in NIH-3T3 cells stably expressing human MT_1 (pEC_{50} = 9.1 ± 0.1, EC_{50} = 0.74 nM, and E_{max} = 59 ± 1%) or expressing human MT_2 (pEC_{50} = 9.0 ± 0.1, EC_{50} = 0.1 nM, and E_{max} = 60 ± 3%) receptors (Fig. 4).

No significant responses (≥50% inhibition or stimulation) were observed in any radioligand receptor binding assay (Table 2) and enzyme assays (Table 3), except for both melatonin receptors MT_1 and MT_2 assays for which approximately 100% inhibition was achieved at 10 nM. Because of overlaps between the ProfilingScreen and DiscoveryScreen panels (Panlabs) and the SpectrumScreen panel (MDS Pharma Services) forty two receptors were tested twice, in some cases with a different assay (i.e. different ligand or different source of receptor); results were consistent in all cases, showing no significant binding affinity for these receptors.

4. Discussion and conclusions

Tasimelteon was shown to have full agonist activity at both melatonin receptors, with greater affinity for the MT_2 receptor as compared to the MT_1 receptor in 2 independent experiments where the human melatonin receptors were expressed in different cell lines. The K_i values of tasimelteon showed numerical differences between cell lines for each melatonin receptor, and between melatonin receptors for each cell line, but remained within the same order of magnitude (Table 1). Such differences are not unexpected for studies with different experimental conditions and may be due to variations in cellular environment (Nelson and Challiss, 2007), receptor reserve (Zhu, 1993) or receptor expression and coupling (Luttrell, 2006), which can affect the K_i estimation (Van et al., 1997). Results were consistent across experiments in demonstrating the binding affinity of tasimelteon for both melatonin receptors, and in indicating a higher MT_1/MT_2 K_i ratio.

MT_1 and MT_2 melatonin receptors are present in several areas of the central nervous system, including the SCN, the pars tuberalis, and the retina, as well as in peripheral tissues such as kidney, pancreas, testes, adrenal cortex, and the immune and cardiovascular systems (Dubocovich and Markowska, 2005). The MT_1 receptor has been implicated in the modulation of neuronal firing (Liu et al, 1997; Jin et al, 2003), cardiac vessel constriction (Krause et al., 1995), reproductive functions (Johnston et al., 2003a; Johnston et al, 2003b), and metabolic functions (Peschke, 2008; Kemp et al., 2002). The MT_2 receptor is believed to mediate circadian rhythm phase-shifting (Liu et al, 1997; Dubocovich et al., 1998, 2005; Hunt et al., 2001), and was recently implicated in the regulation of sleep (Ochoa-Sanchez et al, 2011).

While the binding affinity of tasimelteon for the melatonin receptors has not been determined *in vivo*, the clinical effects of tasimelteon are consistent with the results of the *in vitro* experiments. Tasimelteon has demonstrated the ability to phase-advance the sleep-wake cycle and improve nighttime sleep and daytime sleep (Lockley et al., 2013a, 2013b; Rajaratnam et al, 2009; Torres et al., 2014). Tasimelteon has also demonstrated its ability to phase-advance and entrain the SCN as measured by the circadian rhythm profile of the hormones melatonin and cortisol (Lockley et al, 2013a, 2013b; Rajaratnam et al, 2009).

Tasimelteon had no significant interaction with any other commonly screened receptors or enzyme binding sites tested, including a wide array of receptors of neurotransmitter systems such as dopamine, norepinephrine, serotonin, GABA, acetylcholine, opioid, N-methyl-D-aspartate (NMDA), hypocretin (orexin), and cannabinoid; this finding supports the observation that tasimelteon did not produce signs or symptoms indicative of abuse potential in animal or clinical studies and did not produce withdrawal symptoms after discontinuation of chronic administration (Hetlioz® prescribing information: https://www.hetlioz.com, accessed Aug 27, 2014).

Tasimelteon's receptor binding profile is distinct from that of two other molecules known to bind both melatonin receptors, ramelteon and agomelatine; ramelteon has 8 times lower affinity for the MT_2 receptor than for the MT_1 receptor (Kato et al, 2005), and agomelatine, a non-specific melatonin agonist, has a 4.4 times lower affinity for the MT_2 receptor than for the MT_1 receptor and binds also to several serotonin 5-HT_2 receptors (Millan et al, 2003; Bourin et al., 2004).

The selective and unique receptor binding profile of tasimelteon supports its pharmacological properties as a circadian regulator including in particular its ability to phase advance and entrain circadian rhythms as demonstrated in healthy volunteers (Rajaratnam et al, 2009) and in patients with Non-24 (Lockley et al, 2013a), which leads to improvement in sleep-wake measures and global function in patients affected with Non-24 (Lockley et al, 2013a, 2013b; Torres et al., 2014) .

Table 3
List of enzymes not inhibited by tasimelteon

Enzyme	Tasimelteon (μM)
Calpain	10
Nitric oxide synthase, constitutive	10
Nitric oxide synthase, inducible	10
Protein kinase, EGF receptor kinase	10
Protein kinase, PKCα	100
Protein kinase, PKCβ	100
Protein phosphatase, calcineurin	10

Acknowledgments

The authors would like to thank Drs. Changfu Xiao and Vuk Koprivica for their assistance in the review and analysis of the data, and Drs. Marlene Dressman and Paolo Baroldi for review of the manuscript. This research was funded by Bristol-Myers Squibb and Vanda Pharmaceuticals Inc.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neuropharm.2014.12.004.

References

American Academy of Sleep Medicine, 2005. ICSD-2-The International Classification of Sleep Disorders, second ed. In: Diagnostic and Coding Manual American Academy of Sleep Medicine.
American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, fifth ed., p. 390 (DSM-5).
Bourin, M., Mocaer, E., Porsolt, R., 2004. J. Psychiatry Neurosci. 29, 126–133.
Boyce, P., Barriball, E., 2010. Aust. Fam. Physician 39, 307–310.
Browning, C., Beresford, I., Fraser, N., Giles, H., 2000. Br. J Pharmacol. 129, 877–886.
Cheng, Y., Prusoff, W.H., 1973. Biochem. Pharmacol. 22, 3099–3108.
Czeisler, C.A., Gooley, J.J., 2007. Cold Spring Harb. Symp. Quant. Biol. 72, 579–597.
Davis, S., Mirick, D.K., Stevens, R.G., 2001. J. Natl. Cancer Inst. 93, 1557–1562.
DeLean, A., Munson, P.J., Rodbard, D., 1978. Am. J. Physiol. 235, E97–E102.
Dubocovich, M.L., Markowska, M., 2005. Endocrine 27, 101–110.
Dubocovich, M.L., Yun, K., Al Ghoul, W.M., Benloucif, S., Masana, M.I., 1998. FASEB J. 12, 1211–1220.
Dubocovich, M.L., Hudson, R.L., Sumaya, I.C., Masana, M.I., Manna, E., 2005. J. Pineal Res. 39, 113–120.
Hunt, A.E., Al Ghoul, W.M., Gillette, M.U., Dubocovich, M.L., 2001. Am. J. Physiol. Cell Physiol. 280, C110–C118.
Jin, X., et al., 2003. Mol. Cell Biol. 23, 1054–1060.
Johnston, J.D., Messager, S., Barrett, P., Hazlerigg, D.G., 2003a. J. Neuroendocrinol. 15, 405–408.
Johnston, J.D., et al., 2003b. Proc. Natl. Acad. Sci. U.S.A 100, 2831–2835.
Karlsson, B., Knutsson, A., Lindahl, B., 2001. Occup. Environ. Med. 58, 747–752.
Kato, K., et al., 2005. Neuropharmacology 48, 301–310.
Kemp, D.M., Ubeda, M., Habener, J.F., 2002. Mol. Cell Endocrinol. 191, 157–166.
Knutsson, A., 2003. Occup. Med (Lond) 53, 103–108.
Krause, D.N., Barrios, V.E., Duckles, S.P., 1995. Eur. J. Pharmacol. 276, 207–213.
Liu, C., et al., 1997. Neuron 19, 91–102.
Lockley, S.W., Arendt, J., Skene, D.J., 2007. Dialogues Clin. Neurosci. 9, 301–314.
Lockley, S.W., Dijk, D.J., Kosti, O., Skene, D.J., Arendt, J., 2008. J. Sleep. Res. 17, 207–216.
Lockley, S.W., et al., 2013a. Endoc. Rev. 34. FP26-6.
Lockley, S.W., et al., 2013b. Endoc. Rev. 34. SUN-137.
Lockley, S.W., et al., 2014. Unpublished results (submitted for publication).
Luttrell, L.M., 2006. Methods Mol. Biol. 332, 3–49.
Millan, M.J., et al., 2003. J. Pharmacol. Exp. Ther. 306, 954–964.
Monteleone, P., Maj, M., 2008. Eur. Neuropsychopharmacol. 18, 701–711.
Morikawa, Y., et al., 2005. Scand. J. Work Environ. Health 31, 179–183.
Nelson, C.P., Challiss, R.A., 2007. Biochem. Pharmacol 73, 737–751.
Ochoa-Sanchez, R., et al., 2011. J. Neurosci. 31, 18439–18452.
Peschke, E., 2008. J. Pineal Res. 44, 26–40.
Quera-Salva, M.A., et al., 2014. J. Sleep Res. P433.
Rajaratnam, S.M., et al., 2009. Lancet 373, 482–491.
Ramsey, D.J., Ramsey, K.M., Vavvas, D.G., 2013. Semin. Ophthalmol. 28, 406–421.
Ruger, M., Scheer, F.A., 2009. Rev. Endocr. Metab. Disord. 10, 245–260.
Sack, R.L., Lewy, A.J., 2001. Sleep. Med. Rev. 5, 189–206.
Sack, R.L., et al., 2007. Sleep 30, 1484–1501.
Schernhammer, E.S., et al., 2001. J. Natl. Cancer Inst. 93, 1563–1568.
Schernhammer, E.S., et al., 2003. J. Natl. Cancer Inst. 95, 825–828.
Skene, D.J., Lockley, S.W., Arendt, J., 1999. Biol. Signals Recept. 8, 90–95.
Sliman, J.A., et al., 2014. Sleep 37, A167–A168.
Swick, T.J., et al., 2014. 167th Annual Meeting, New York, NY. American Psychiatric Association, pp. 419–420.
Torres, R. , et al., 2014. 167th Annual Meeting. American Psychiatric Association, New York, NY, p. 483.
Turek, F.W., 2007. Int. Clin. Psychopharmacol. 22 (Suppl. 2), S1–S8.
Uchiyama, M., Lockley, S.W., 2009. Sleep Med. Clin. 4, 195–211.
Van, D.B., Dijcks, I.,F.A., Vanderheyden, P., Vauquelin, G., Oortgiesen, M., 1997. J. Pharmacol. Exp. Ther. 281, 1113–1119.
Vyas, M.V., et al., 2012. BMJ 345, e4800.
Zhu, B.T., 1993. J. Pharmacol. Toxicol. Methods 29, 85–91.